REMARKS

The Official Action of December 17, 2003 has been carefully considered and reconsideration of the application as amended is respectfully requested.

The specification has been amended to provide an Abstract as requested by the Examiner at paragraph 7 of the Official Action.

The claims have been amended with the recitations formerly in claims 1 and 5 having now been inserted into new claim 23. New claims 24-43 have been added more completely to define the subject matter which Applicants regard as their invention. The recitations in new claims 24-25 and 27-42 correspond to the recitations formerly in claims 2-4 and 6-20. The recitations in new claim 26 draw support from the specification as filed at, for example, page 3, last paragraph. The recitations in new claim 43 draw support from the specification as filed at, for example, page 6, lines 18-21.

The claims as rewritten are respectfully believed to be free of the informalities noted at paragraph 8 of the Official Action and are otherwise believed to be sufficiently definite to satisfy the dictates of 35 USC 112, second paragraph. Similarly, the claims as rewritten are respectfully believed to be patentable over the prior art (Bennett) cited at paragraph 11 of the Official Action. It is noted in this respect that the cited art was not applied against the subject matter formerly in claim 5 which has now been incorporated into the main claim. As discussed previously, in Bennett, a wetting agent is applied to assist in a seed sterilization step, by improving the wetting of the seed with bleach. The wetting agent is removed before the seed is contacted with the *Agrobacterium*. There is nothing in Bennett that would show or

suggest the invention as defined in the claims now on file, including the claimed contacting with a wetting agent during vacuum infiltration.

Claims 1-20 stand rejected under 35 USC 112, first paragraph, because the specification is allegedly not enabling for the invention as claimed. Applicants respectfully traverse this rejection.

Applicants submit herewith a Declaration under 37 CFR 1.132 signed by one of the co-inventors showing that it would take no more than routine (i.e. not undue) experimentation for one of skill in the art to practice the invention as now claimed. In this connection, the specification need only be enabling for one of skill in the art and, as pointed out in the Declaration, as of the application filing date, those of skill in the art had experience with (a) vacuum infiltration to introduce microorganisms into plant cells and (b) the use of surfactants to facilitate the introduction of material into plant cells without damaging the plant cells. Thus, once it is also disclosed in the present specification that the use of a wetting agent/surfactant in connection with vacuum infiltration could be used to transform germinating plant seed with *Agrobacterium*, one of skill in the art could practice the invention as claimed using only routine experimentation with techniques well known to those of skill in the art.

It is respectfully submitted that the Declaration successfully rebuts the points raised by the Examiner at paragraphs 9 and 10 of the Official Action. Specifically, with respect to the alleged need for undue experimentation to determine suitable conditions for vacuum infiltration, the Declaration shows that, as of the application filing date, it was routine in the art to test a vacuum infiltration method at various vacuum conditions, for various times and for various development phases of the seed.

While the conditions described in Example 1 of the specification gave optimum results, other conditions would also work (although perhaps less effectively). The declaration shows that one of skill in the art could routinely ascertain when the vacuum would be high enough to displace air from the cells with *Agrobacterium* without rupturing the cells.

With respect to the alleged need for undue experimentation in the selection of other suitable wetting agents/surfactants, the Declaration shows that one of skill in the art would have been able routinely to select a surfactant that is not toxic to the plant material, such as those surfactants previously used to introduce other material or bacteria into plants. In this connection, Applicants respectfully note that the claims are no longer so broad as to cover any and all wetting agents/surfactants (see *Wands* factor cited in the Official Action at page 10, penultimate paragraph: "the breadth of the claims"), but are limited by a functional recitation to those that would facilitate or enhance penetration and transformation of the germinating plant seed by the *Agrobacterium* strain. Obviously, this recitation excludes wetting agents/surfactants that would be toxic to the plant seed and this recitation must therefore be taken into account when considering the question of enablement (see MPEP Section 2164.01(c): "When a compound or composition claim is limited by a particular use, enablement of that claim should be evaluated based on that limitation.") Moreover, the fact that those of skill in the art had experience with application of such wetting agents/surfactants in suitable dosages must also be taken into account (Id: ". . . it is not necessary to specify the dosage or method of use if it is known to one skilled in the art that such information could be obtained without undue experimentation.")

In addition to the above, it is respectfully submitted that there is another of the *Wands* factors that must be considered in determining the question of enablement, namely: "the quantity of experimentation" (see MPEP Section 2164.06). The test is not merely quantitative since a considerable amount of experimentation is permissible, if it is merely routine or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed. Id.

In the present case, Applicants respectfully note that the specification provides actual working examples (another *Wands* factor) that those of skill in the art could routinely follow to ascertain whether any given surfactant would work in the claimed method to facilitate or enhance penetration and transformation of germinating plant seed by *Agrobacterium*. Given the routine nature of this experimentation and the fact that one of skill in the art need only duplicate the experimentation set forth in the specification to test the claimed method with any given surfactant, it is respectfully submitted that it cannot be considered that the amount of experimentation needed to practice the claimed invention with wetting agents/surfactants other than that exemplified would be "undue".

With respect to the Examiner's contention at the end of paragraph 10 of the Official Action that the specification is only enabling to the extent the claims are limited to germinating soybean seed, Applicants respectfully submit that the Examiner has not met the USPTO burden of setting forth a reasonable basis to question the accuracy of Applicants' presumptively accurate disclosure (see MPEP Section 2164.04 and note that the specification at the last paragraph on page 3 describes the

use of the claimed method with a wide variety of other plants). In any event, the claims have now been limited by functional recitation to germinating plant seed that are susceptible to transformation with *Agrobacterium* and to an *Agrobacterium* strain that is capable of transforming the germinating plant seed. Moreover, the Declaration submitted herewith shows clearly that one of skill in the art can practice the claimed method with other plant seed without undue experimentation.

In view of the above, it is respectfully submitted that the specification is enabling for the full breadth of the claims as amended and that the rejections under 35 USC 112, first paragraph should be withdrawn. Since all objections and rejections of record have been overcome, it is respectfully considered that the application is in allowable form. An early notice of allowance is earnestly solicited and is believed to be fully warranted.

Respectfully submitted,

CLIFFORD J. MASS
LADAS & PARRY
26 WEST 61ST STREET
NEW YORK, NEW YORK 10023
REG. NO.30,086(212)708-1890

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: Jacoba Adriana DE RONDE
Serial No.: 09/807,391 Group No.:
Filed: October 14, 1999 Examiner.:
For: TRANSFORMATION PROCESS

Attorney Docket No.: U 015065-1

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 CFR 1.132

I, Jacoba Adriana De Ronde, declare as follows:

1. I am a co-inventor of the invention described and claimed in US patent application serial number 09/807,391 ("the application"). I make this declaration in support of the application. My technical background and experience are as shown on my curriculum vitae, which is annexed hereto as Exhibit 1.

2. I understand that the Examiner of the application has considered that it would require "undue trial and error experimentation" for one of skill in the art to practice the invention described and claimed in the application with (a) surfactants or wetting agents other than those exemplified in the application or (b) vacuum infiltration under conditions other than those exemplified in the application or (c) plant seed other than soybean seed. However, as discussed below, as of the filing date of the application, those of skill in the art had experience with vacuum infiltration for transforming certain plants (*Arabidopsis thaliana*) with *Agrobacterium* and with the use of surfactants to enhance penetration of bacteria and other materials into plants in general. As also discussed below, based on the disclosure in the specification of the present application, those of skill in the art could have routinely determined suitable surfactants, vacuum infiltration conditions, and plant seed for use in the method described and claimed in the application using the specification and the examples provided therein as a guide.

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3. As of the filing date of the application, 14 October 1999, one of ordinary skill in the art to which the invention pertains would have had an advanced degree in molecular or plant biology and experience in transforming plants with *Agrobacterium* by a variety of techniques, including with vacuum infiltration (with respect to *Arabidopsis thaliana*). One of skill in the art would have had access to or familiarity with the publications cited below.

4. Vacuum Infiltration

As of 14 October 1999, vacuum infiltration was a known technique for the introduction of material into plants. For example, in 1993, Bechtold *et al* described a procedure for "*Agrobacterium* vacuum infiltration" for the transformation of *Arabidopsis thaliana* with *Agrobacterium*. US Patent 5,415,672 describes the use of vacuum infiltration to force into seeds both pathogenic microorganisms ('672 patent at column 2, lines 14-29) and non-pathogenic microorganisms ('672 patent at column 9, lines 17-29, and Example 2 at column 10). Indeed, as of 14 October 1999, those of skill in the art were familiar with vacuum infiltration and with techniques for determining optimal conditions, including pressure conditions, for vacuum infiltration that would facilitate the delivery of bacteria to plants and to plant seed. Moreover, I and my co-inventors tested the method described in the application for the transformation of plant seed at various vacuum conditions, for various times and for various development phases of the seed, and we describe the optimal conditions in Example 1 of the application. Other conditions will also work, although less effectively. For example, at an absolute pressure which is too high, the process would take too long or would provide insufficient transformation of the seed to make it worthwhile. Nevertheless, the process would still be operable so long as the vacuum is not so high that the seed is damaged and is sufficiently high to displace air from the cells with *Agrobacterium* thereby to transform the seed. Given the guidance provided in the example in the specification and the knowledge of those of skill in the art as of the filing date of the application, those of skill in the art could routinely determine vacuum infiltration conditions that would provide for an operable process.

5. Wetting Agents/Surfactants

As of 14 October 1999, the use of surfactants to facilitate or enhance the

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penetration of bacteria or other materials into plant cells was known to those of skill in the art. For example, prior to October 1999, US Patent 5,415,672 describes the use of surfactants to aid in the delivery of clavibacter micoorganisms to plant seed (see '672 patent at column 7, lines 22-51: "The surfactants aid in permitting the suspension of the microorganisms and the carrier to penetrate microscopic cracks and fissures in the hard, outer seed coat so that the microorganisms end up within the coat."). Other patents describe the use of surfactants to facilitate or enhance the penetration of other material into plant tissue. E.g., US Patent 6,667,276 (known effect of pesticides increased with surfactants); US Patent 6,605,145 (efficient application of agricultural products dependent on the dynamic surface tension properties of formulation); and US Patent 4,219,965 (surfactant used to enhance penetration of oleaginous material into plant for frost damage prevention in plant tissue). Accordingly, as of 14 October 1999, those of skill in the art could routinely select wetting agents or surfactants that could be used with plant seed without harming the plant seed. Indeed, any surfactant which is suitable for use with plant material should be usable in the method described in the application and a person of skill in the art could routinely select a suitable surfactant and test it simply by replicating the examples described in the application. Since the surfactants are known and have previously been used with plants as, for example, pesticides, suitable dosages would also have been known to those of skill in the art.

6. Seed Other Than Soybean Seed

As discussed above, as of October 14 , 1999, techniques for introduction of materials into plant seed by vacuum infiltration were well known to those of skill in the art. Also known to those of skill in the art were techniques for facilitating or enhancing penetration of material into plants with wetting agents/surfactants. What was not known was whether wetting agents/surfactants could be used in combination with vacuum infiltration to transform germinating plant seed with *Agrobacterium*. This disclosure is only supplied by the present application but, once it is shown that the use of wetting agents/surfactants when used in conjunction with vacuum infiltration could be used to transform germinating plant seed such as soybean seed (Examples 1-4 in the application) and lupin seed (Example 5 in the application), one of skill in the art could routinely apply the techniques already known to him or her in conjunction with the techniques described in the application to transform a wide variety of germinating plant seed. I have now confirmed this with the following experimentation of which I have

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firsthand knowledge.

Protocol

The plant expressions vector pCambia 3301 vir G in *Agrobacterium tumefaciens* strain LBA 4404 was used for transformation on lupin, wheat, canola and sunflower. This binary vector contained the following downstream from the right T-DNA border: ß-glucuronidase reporter gene (GUS INT) cauliflower mosaic 35S promoter, bar gene and the left T-DNA border. Seeds of lupin, wheat, canola and sunflower were surface sterilized with a 3.5% solution of sodium hypochlorite for 5 minutes. After three rinses with sterile distilled water the seeds were placed on sterile agar/water medium in petridishes and incubated at 29°C for 1 to 3 days, until the seed were partially geminated.

The *Agrobacterium* was cultured from fresh plates into 100 ml Luria-Bertani broth (LB) pH 7.00 supplemented with 150 µg ml^{-1} rifampicin and 100 µg ml^{-1} kanamycin at 27°C until the $A_{600} = 0.5$. Acetosyringone (0.01 $mgml^{-1}$) was added to the *Agrobacterium* culture at this stage and cultured for another 12 hours. Cells were centrifuged at 10 000 rpm for 15 - 20 minutes at 10°C. The bacterial pellet was resuspended in 100 - 400 ml PO_4 buffer (depend on the size of the pellet) supplemented with 0.1 % Break-Thru.

The partially germinated seed were carefully added to the *Agrobacterium* solution into an erlenmeyer flask. The erlenmeyer's top was covered with parafilm before the erlenmeyer flask was inserted into a vacuum compartment of a freeze drier and vacuum was applied. The seed was vacuum infiltrated with the *Agrobacterium*/wetting agent suspension for 20 minutes at ± -80 KPa. During the 20 minute vacuum, little bubbles escaped the whole time from the seeds into the solution. There after the vacuum was quick released. After the transformation treatment, the seeds were thoroughly washed in PO_4 buffer before planted out directly in the greenhouse. The seed was planted in a soil mix of 5:3:3 cultera: soil: vermiculite in small pots at day/night temperatures of 28/18°C under a daily irrigation system. Leaf material was harvested from the putative transformants (T0 and T1) and the control plants once. One milliliter of GUS extraction buffer (50mM $NaPO_4$, pH 7; 10mM EDTA, pH 8; 10mM mercapto ethanol) was added to the leaf tissue with 2M of X-GLUC. Leaves of the putative transgenics (T0 and T1)

were sprayed with Ignite® and left for 3-5 days on the plant for observation. PCR analysis was performed on surviving plants.

Results

Leaf tissue of the non transgenic and transgenic plants was incubated together with an X-GLUC solution in an eppendorf to test for *gus* expression. The results were very promising as the leaves of some putative transgenics turned blue and the non-transgenics did not. Six weeks old putative transgenics (T0) were tested for *bar* expression. The plants were sprayed with Ignite® to see what the effects on the plants were. The results were that some of the plants survived. The same plants that tested positive for *gus* survived the Ignite® tests. The PCR proved that the *gus* and *bar* genes were present in the plants that survived. The plants identified with resistance to Ignite® were left to make seed. Some T1 plants tested positive for *bar* and *gus* expression. The transformation success varied between experiments, crops and cultivars, but the lowest success rate observed with the pCambia 3301 vector was 8%.

7. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity or the application of any patent issued thereon.

Date: 3 May 2004

Jacoba Adriana De Ronde

EXHIBIT 1

CURRICULUM VITAE: KOBIE DE RONDE

Work address	Agricultural Research Council Roodeplaat Vegetable and Ornamental Plant Institute Abiotic stress unit Private Bag X293 Pretoria 0001
Telephone number	27 12 841 9775/ 841 9643
Fax number	27 12 8080844

PERSONAL DATA

Surname:	De Ronde
First name:	Jacoba Adriana (Kobie)
Identity number:	6502240090083
Sex:	Female
Marital status:	married
Drivers licence:	Yes, code 08
Nationality:	South Africa
E mail address	kobie@vopi.agric.za
Language	Afrikaans: speak, read, write and understand English: speak, read, write and understand

EDUCATIONAL QUALIFICATION:

Universities attended:	University of Pretoria (1986) University of Natal (1994) University of Natal (2001)

Degrees obtained and major subjects

1. BSc Agric (Genetics), 1987, Pretoria University
2. MSc (Molecular aspects on heat tolerance in cotton), 1994, Natal University, Pietermaritzburg

Promoter: Dr WA Cress

3. PhD (Proline biosynthesis in transgenic soybean plants) 2001, Natal University

Promoters: Dr WA Cress, Prof J van Staden

EMPLOYMENT INFORMATION

Employment Agricultural Research Council
Roodeplaat Vegetable and Ornamental
Research Institute
(Desember 1986 - +)

Main job function

1. Project Management (August 1998 - current)

Responsible for management and supervising of research in drought tolerance and heat tolerance in agricultural crops. The crops involved include: Maize, Potato, Cotton, Pigeon peas, Cowpeas, Soybean, Amaranthus spp, Bambara groundnut and *Eucalyptus* spp. Different approaches to drought tolerance include:

1. physiological studies: leaf water potential, relative water potential, proline studies and enzymes involved in proline pathway, triphenyltetrazolium chloride test (viability assay), chlorophyll fluorescence studies, water use efficiency studies, polyamine analysis and enzymes involved in polyamine pathway, CuZn superoxide dismutase, glutathione reductase, ascorbate peroxidase and peroxidase measurements
2. anatomical studies: root architecture boxes, wooden boxes
3. mutation technology: Improvement of *Amaranthus tricolor, Vigna unguicalata* and *Vigna subterranea* through mutation techniques
4. transformation studies: potato, sunflower, lupines and soybean.
5. molecular studies: PCR, southern blot, molecular marker technology

2. Research

2.1. I was involved in research on heat and drought tolerance in cotton. The project aimed to determine the most effective methods of screening for such tolerance, as well as the interactivity between heat and drought stress. A number of techniques were evaluated including: triphenyl tetrazolium chloride reduction viability tests, free proline determinations, chlorophyll fluorescence measurements, polyamine analysis, heat shock protein synthesis, iso-enzymes, protein-DNA binding, western blot analysis, cDNA libraries, CuZn superoxide dismutase, glutathione reductase, ascorbate peroxidase, peroxidase. The project was designed to facilitate selection of cotton for heat and drought tolerance and identify tolerant and susceptible varieties for use in future genetic studies.

2.2. Currently I am involved in research on the role of proline in soybean as a result of drought stress. The aim of this research is to develop a system in which the role of

proline biosynthesis in response to drought stress in soybean may be clarified by the use of sense and antisense gene technology.

This includes: biotechnology studies: vacuum infiltration mediated transformation of soybean seed with *P5CR* gene

Molecular studies: Southern blot, PCR, DNA analysis

Physiological studies: proline studies and enzymes involved in proline pathway, viability assays, NADP/ NADPH ratios, chlorophyll fluorescence measurements, CuZn superoxide dismutase and glutathione reductase analysis.

Greenhouse trials as well as rainout shelters were used in he drought stress application

3. **Training**

Participate in various international-training courses

Training of scientists/students (small groups or one to one basis) in molecular and physiological techniques

As a regional co-ordinator for AFRA, I am responsible for:

- organisation of training courses for drought tolerance, biotechnology and mutation technology
- organising short scientific visit of scientists as well as fellowships (up to 3 months)
- organising site visits for scientists and liaison officers

Courses organised:

ICRO-UNESCO course on screening methods for drought tolerance in food crops, 2000, ARC-Roodeplaat, Pretoria

AFRA Regional Training Course: Improved mutation, *in vitro* culture and drought screening techniques for the improvement of African crops, 15 October 2001, ARC-Roodeplaat, Pretoria

AFRA RAF/5/050 Project Co-ordination Meeting: Increasing Production of Nutritious Food through Mutation Breeding and Biotechnology, 10-14 March 2003, Rietfontein, Pretoria

Third FAO/IAEA research coordination workshop: Genetic improvement of underutilized and neglected crops in LIFDCS through irradiation and related techniques, 19 – 23 May, 2003, Rietfontein, Pretoria

Fellowships organised in 2002:

Ms Miriam Kinyua from Kenya, Mr William K Chishimba from Zambia, Mr Paul Mumba from Zambia

External examiner: thesis

2001: MSc: AZ Lemma, Characterization of wheat cultivars for drought stress tolerance, University of Orange Free State

2002: PhD: SF Kebede, Analysis of drought tolerance in durum wheat genotypes, University of Orange Free State

External examiner: university exams

2003: University of Pretoria, BOT 354

Refereed papers and proposals

2001: Physiologia plantarum article: Role of proline accumulation upon osmotic stress and of D1-pyrroline-5-carboxylate synthetase in the salinity and drought resistance of three rice cultivars" by DT Hien, M Jacobs, LV Son and NH Roosens

2001: Project proposal NOPO: Prof A Oberholster, Utilizing a proteinase inhibitor in peanut

2002: SA Journal of Science article: Lamainski, Gray, Mycock, Blatch, Groll, Rey. The green fluorescence protein as a reporter of transformation in cassava.

2002: NRF proposal: University Free State Maartens, H, Environmental stress in crops

2002: The Southern African Forestry Journal article: Rolanda CA and Little KM....Using chlorophyll fluorescence to determine stress in *Eucalyptus grandis* seedlings.

Merit awards

Obtained the merit award from the Protein Research Council in 2002 for the best PhD thesis in 2001.

Publications

1994

De Ronde, J.A , 1994. MSc - Molecular aspects on heat tolerance in cotton, Natal University

1995

De Ronde, J.A., Van der Mescht, A. and Cress, W.A., (1995). The biochemical responses of six cotton cultivars to heat stress. *S. Afr. J. Sci.* 91: 363-366.

1997

De Ronde, J.A. and Van der Mescht, A., 1997. Utilization of 2,3,5-Triphenyltetrazolium chloride reduction as a measure of the interaction between drought tolerance simulation and heat tolerance in cotton. *S. Afr. J. Science* 93: 431-433

Van der Mescht, A, De Ronde, J.A. , Van der Merwe, T., Laurie, R., Bester, C. and Wenzel, C., 1997. Evaluation of chlorophyll fluorescence as a measure of drought tolerance in *Eucalyptus grandis*. Proceedings of the IUFRO conference on silviculture and improvement of Eucalypts. Vol 4: 117-124.

1998

Van der Mescht, A., De Ronde, J.A. and Rossouw, F.T. 1998. Cu/Zn Superoxide dismutase, glutathione reductase and ascorbate peroxidase levels during drought stress in potato. *S. Afr. J. Sci 94: 496-498.*

Van der Mescht, A., De Ronde, J.A., Van der Merwe, T. and Rossouw, F.T. Changes in free proline concentrations and polyamine levels during drought stress in potato. *S. Afr. J.Sci: 94: 347-350*

Van der Merwe, T, L. van Staden, A. van der Mescht and J.A. de Ronde, 1998. Triphenyl tetrazolium chloride: a new method to determine temperature tolerance in roses. *Journal of the Southern African Society for Horticultural Science*. 18 (1) : 21-23

1999

Van der Mescht, A. , De Ronde, J.A. and Rossouw, F.T., 1999. Chlorophyll fluorescence and chlorophyll content as a measure of drought tolerance in potato. *S. Afr. J. Sci* 95: 407-412

2000

De Ronde J.A, Van Der Mescht, A and Steyn, H.S.F. 2000 Proline accumulation in response to drought and heat stress in cotton. *African Journal of Science*, 8 (1) :85-91

De Ronde, J.A., Spreeth, M.H. and Cress, W.A., 2000. Effect of antisense L- Δ^1- pyrroline-5-carboxylate reductase transgenic soybean plants subjected to osmotic and drought stress. *Plant Growth Regul.* 32: 13-26

De Ronde, JA, Van der Mescht, A, Laurie, RN, Spreeth, MH and Cress, WA, Molecular approach to drought and heat tolerance for selected crops. Water Research Commission 479/1/99 ISBN 1 86845 556 4

2001

De Ronde, J.A. 2001, Proline biosynthesis in transgenic soybean plants Universiteit Natal, Pietermaritzburg, PhD thesis

De Ronde, JA, WA Cress and J Van Staden, 2001 Interaction of osmotic and temperature stress on transgenic soybean. *S A J Botany 67:655-660*

De Ronde, JA , WA Cress and A van der Mescht, 2001 Agrobacterium mediated transformation of soybean (Glycine max) seed with the B glucuronidase marker gene. *S A J Science 97: 421-424*

2003

JA de Ronde, 2003. Regional designated centres in the field of agriculture. Mutation breeding and biotechnology. p 9-12 AFRA , Designated centres, IAEA

JA de Ronde, 2003. Biolines 50, Africa Bio

Submitted

Van der Mescht, A. , De Ronde, J.A. , Slabbert, M.M. , Murray S. , Oelofse, D. and Rossouw, F.T. Enhanced drought tolerance in transgenic potato expressing the A*rabidopsis thaliana* Cu/Zn superoxide dismutase gene. *S. Afr. J. Sci*

Van der Mescht, A., De Ronde, J.A., Van der Merwe, T., Daniels, C.L. and Rossouw, F.T. A comparison of drought stress and heat stress in the leaves and tubers of 12 potato cultivars. *S. Afr. J. Sci*

De Ronde Jacoba A., Cress William A., Krüger Gert H.J., Strasser Reto J. and Van Staden Johannes Photosynthetic response of transgenic soybean plants containing the *P5CR* gene during heat and drought stress *J Plant Physiol proofs*

De Ronde JA., Cress William A., and Van Staden J. Phenotypic evaluation for drought tolerance of transgenic soybean. *SAJ Plant Soil*

De Ronde JA, RN Laurie, T Caetano, MM Greyling & I Kerepesi Comparative study between transgenic and non transgenic soybean lines proved transgenic lines to be more drought tolerant. *Euphytica*

Papers

1995

De Ronde, J. A. , 1995. Molecular and physiological aspects of heat tolerance in cotton. Heat stress work shop, Rand Afrikaans University, Johannesburg

1996

De Ronde J.A., 1996. Regeneration and transformation of soybean. Protein research Trust workshop, Roodeplaat

1997

De Ronde, J.A. and Cress, W.A., 1997. *Agrobacterium* mediated transformation of soybean. Biotegnologie simposium, Roodeplaat, Pretoria

De Ronde, J.A. and Van der Mescht, A., July 1997. Physiological approach to environmental stress. Agricultural Institute of academy, Martonvasar, Hungary

De Ronde, J.A. and Van der Mescht, A., July 1997. Physiological measurement of drought and heat tolerance. Agricultural Biotechnology Centre, Godollo, Hungary.

De Ronde, J.A. and Van der Mescht, A., July 1997. Strategy in solving the environmental stress problem in South Africa. Biological Research Centre, Hungarian academy of science, Szeged, Hungary.

Van der Merwe, T., Van der Mescht, A., Van Staden, L and De Ronde, J.A., 1997. Evaluation of 2,3,5-triphenyl tetrazolium chloride reductions as a measure of drought and heat tolerance in *Cajanus cajan*. SASHS, Nelspruit.

1998

Daniels, C., Van der Mescht, A., De Ronde, J.A. and Van der Merwe, T., 1998. Heat and drought tolerance as measured by 2,3,5- triphenyl tetrazolium chloride reduction, is organ specific in potato. S.A. Crop Science Congress, Natal.

De Ronde, J.A. and Cress, W.A., 1998. *Agrobacterium* mediated transformation of soybean (*Glycine Max*) with the P5CR gene. S.A. Crop Science Congress, Natal.

De Ronde, J.A., Van der Mescht, A. and Cress, W.A., 1998. Agrobacterium mediated transformation of soybean in manipulation of proline. Internasional stress symposium. Rand Afrikaans University (Invited lecture)

De Ronde, J.A., Van der Mescht, A., Van der Merwe, T and Cress, W.A., 1998. How do plants respond to heat stress? Botany departement, Pretoria University. Invited lecture.

Van der Mescht, A., De Ronde, J.A., Spreeth, M., Slabbert, R., Laurie, R.N. and Van der Merwe, T., 1998. Physiological approach to drough tolerance. Invited paper, Departement of botany, University of Pretoria.

Van der Mescht, A, Laurie, R.,Van der Merwe, T., De Ronde, J.A. and Bester, C., 1998. Chlorophyll fluorescence as a measure of drought tolerance in *Eucalyptus grandis*. S.A. Crop Science Congress, Natal.

Van der Merwe, T., Daniels, C., De Ronde, J.A. and Van der Mescht, A., 1998. The role of proline and polyamine accumulation in pigeon peas during drought stress. S.A. Crop Science Congress, Natal.

Van der Merwe, T., Van der Mescht, A., De Ronde, J.A., Van Staden, L. and Rossouw, F.T., 1998. Chlorophyll fluorescence as a measure of drought tolerance in potato. Plant Breeders Symposium.

1999

Cress W.A and J.A. de Ronde, December 1999. Proline and stress in Soybean. Centro de investigation Cientifica de Yucatan, Merida, Mexico

De_Ronde, J.A, September 1999. The effect of enhance proline synthesis in soybean, with special reference to drought stress, comparing antisense and sense technology. Soybean workshop, Pretoria.

De_Ronde , J.A., W.A. Cress and J. van Staden, November 1999. Effect of under and over expression of the P5CR gene in transgenic soybean on chlorophyll fluorescence. 15th RUPGD, University Natal Pietermaritzburg.

2000

Caetano T. and J.A. de Ronde, 2000. Drought tolerance screening for *Cajanus cajan* using various laboratory screening methods.. 26th Annual Conference of the South African Association of Botanists, Potchefstroom

Caetano T. and J.A. de Ronde, 2000. Drought tolerance screening for *Cajanus cajan* using various laboratory screening methods.. Combined Congress, Bloemfontein

De Ronde J.A. and W.A. Cress, 2000. The under expression of a proline gene in transgenic soybean plants subjected to an osmotic stress. 26th Annual Conference of the South African Association of Botanists, Potchefstroom

Laurie RN. and De Ronde, JA. 2000. Various methods available to screen for drought tolerance. Hungarian Research Institute, Martonvasar, Hungary

Laurie, R. N., De Ronde, J.A. and Van der Mescht, A. Use of the 2,3,5-triphenyltetrazolium chloride (TTC) assay to evaluate heat and drought tolerance in sweet potato. 26th Annual Conference of the South African Association of Botanists, Potchefstroom

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